Small intestinal bacterial overgrowth in patients with rheumatoid arthritis

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Abstract

***Objectives*—To examine the microflora of the upper small intestine in patients with seropositive rheumatoid arthritis (RA) using a combination of microbial cultivation and tests for microbial metabolic activity.**

***Methods*—Twenty five patients with seropositive RA, 12 achlorhydric control subjects, and 11 control subjects with normal gastric acid secretion were investigated. Disease activity was evaluated in the patients with RA by three different indices. Eight (32%) of the patients with RA had hypochlorhydria or achlorhydria. The acid secetory capacity was determined with pentagastrin stimulation. A modified Crosby capsule was used to obtain biopsy specimens and samples of intestinal fluid from the proximal jejunum; aerobic and anaerobic microbial cultivation of mucosal specimens/ intestinal fluid was carried out, and gas production and microflora associated characteristics in jejunal fluid were determined. Additionally, a bile acid deconjugation breath test was performed.**

***Results*—Subjects with at least one of the following findings were considered to have bacterial overgrowth: positive bile acid deconjugation test; growth of Enterobacteriaceae; positive gas production; or low tryptic activity. By these criteria half of the patients with RA with hypochlorhydria or achlorhydria and half of the achlorhydric controls had bacterial overgrowth. Thirty five per cent of the patients with RA with normal gastric acid secretion had bacterial overgrowth compared with none of the normal controls. Disease activity indices and rheumatoid factor titres were significantly higher in patients with RA with bacterial overgrowth than in those without.**

***Conclusions*—A high frequency of small intestinal bacterial overgrowth was found in patients with RA; it was associated with a high disease activity and observed in patients with hypochlorhydria or achlorhydria and in those with normal acid secretion.**

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Alterations in the intestinal microflora may be important in the pathogenesis of rheumatoid arthritis (RA),[1–4] a disease in which even specific micro-organisms have been implicated.[1 5] In this context it is noteworthy that the pathogenesis of jejunoileal bypass arthritis, which may be indistinguishable from RA,[6] is associated with bacterial overgrowth of a faecal type of microflora resulting in the formation of immune complexes.[7] Moreover, peripheral arthritis is a common manifestation of gastrointestinal infections including Whipple's disease, further supporting a possible causal relation between the development of arthritis and qualitative or quantitative changes in the microflora of the gastrointestinal tract.

The colonic microflora is composed of more than 500 bacterial species which interact with host and environmental factors.[8 9] Continuously changing interactions and variations in the concentrations of the bacteria as well as in their metabolic activity[9] make an accurate determination of the characteristics of the microflora difficult. Thus at present no single technique can give a comprehensive picture of the microbial status of the host and microbiological studies in vivo should preferably use a combination of techniques.

The aim of this study was to examine the microflora in the upper small intestine in patients with seropositive RA by using a set of tests that measure microbial metabolic activity combined with bacterial cultivation. In addition, an effort was made to correlate bacterial overgrowth to patient and disease characteristics.

Subjects and methods

CHARACTERISTICS OF PATIENTS AND CONTROLS

Twenty five patients with RA defined according to the criteria of the American Rheumatism Association[10] were included in the study (table 1). All patients were seropositive for rheumatoid factor and had erosive joint disease with symmetrical and peripheral arthritis. Six patients had subcutaneous nodules and one had secondary Sjögren's syndrome. The median duration of the disease was eight years (range four months to 38 years) as evaluated from the onset of symptoms. The physical disability of the patients was graded I to IV according to Steinbrocker *et al*,[11] 10 patients being rated grade II and 15 grade III. At the time of the study three patients were not receiving drugs, and 21 were continuously taking non-steroidal anti-inflammatory drugs (NSAIDs); of these 19(90%) had been treated with NSAIDs for more than four months with a median duration of two years (eight months

Table 1 Demographic characteristics and ABO blood group of patients with rheumatoid arthritis (RA), achlorhydric controls (control I) and healthy volunteers with normal gastric acid secretion (control II). Values are median (interquartile range) or No (%). There are no significant differences between the groups

	All patients with RA (n=25)	*Hypochlorhydric or achlorhydric patients with RA (n=8)*	*Patients with RA with normal gastric acid secretion (n=17)*	*Control I (n=12)*	*Control II (n=11)*
Demographic characteristics					
Age (years)	60(47–64)	63(61–64)	56(44–62)	65(48–69)	55(44–62)
Female sex	17(68)	6(75)	11(65)	8(67)	8(73)
ABO Blood group					
O	9(36)	3(38)	6(35)	3(25)	3(27)
A	8(32)	3(38)	5(29)	4(33)	6(55)
B	5(20)	1(13)	4(24)	3(25)	1(9)
AB	3(12)	1(13)	2(12)	2(17)	1(9)

to nine years); the three most common drugs were naproxen (median dose 1000 mg), ibuprofen (median dose 1800 mg), and indomethacin (median dose 100 mg). Nine patients were taking corticosteroids (median dose 5 mg prednisolone). Four patients had never taken disease modifying antirheumatic drugs (DMARDs), and another six had not taken DMARDs during the past year. Of the 15 patients treated with DMARDs during the past year, nine stopped treatment more than three months before the study and six one month before the study.

Owing to the high prevalence of atrophic gastritis[12] and hypochlorhydria [13] in patients with RA, conditions which are associated with a pathological flora in the small intestine,[14] two age and sex matched control groups were used. Twelve patients with known achlorhydria were included in control group I (table 1). Seven of these patients had pernicious anaemia (treated with vitamin B-12), five had hypothyroidism (substituted with thyroxine), and two had alopecia. None of the patients had symptoms or signs of spinal or peripheral inflammatory joint disease and none had received NSAIDs. Control group II consisted of 11 healthy volunteers who denied even occasional use of NSAIDs (table 1).

None of the patients or controls had been treated with antibiotics or H_2 receptor antagonists or omeprazole during the month before the study, and none had an operation in the upper gastrointestinal area.

Rheumatic disease activity and functional capacity were assessed by the determination of the Riel index,[15] the Ritchie articular index,[16] the health assessment questionnaire (HAQ),[17] and by laboratory markers of inflammation: erythrocyte sedimentation rate, C reactive protein, and orosomucoid. Circulating immune complexes in serum,[18] rheumatoid factor, serum levels of IgA, ABO blood group, blood haemoglobin level, and thrombocyte count were determined. All examinations were performed after an overnight fast.

The study was approved by the Karolinska Hospital ethical committee. Informed consent was given by the patients and controls.

GASTRIC SECRETION TEST

Before the test, the mouth was rinsed with an antiseptic solution containing ascorbic acid, sodium percarbonate, and copper sulphate (Ascoxal, Astra, Södertälje, Sweden). The stomach was intubated and saliva was continuously aspirated. After three 15 minute collections of basal gastric secretion, pentagastrin 6·0 μg/kg (Peptavlon, ICI, Macclesfield, United Kingdom) was given subcutaneously. The stimulated gastric secretion was collected for four 15 minute periods. Samples of the gastric contents were aspirated for aerobic and anaerobic microbial cultivation with a sterile syringe 15 minutes before and 45 minutes after the pentagastrin injection. The volume and the acidity of the samples was determined. The basal gastric acid secretion was expressed as the acid output during the 30 minutes before stimulation, and the peak acid output as the sum of the two highest consecutive periods after pentagastrin administration.

MICROBIOLOGICAL INVESTIGATIONS

Collection of specimens

A Crosby capsule was used to take a biopsy specimen from the proximal jejunum, immediately distal to the Treitz ligament. A polyethylene catheter (inner diameter 1·50 mm, outer diameter 2·10 mm) was attached to the catheter holding the capsule to aspirate the intestinal contents. All samples were collected by the same examiner 12–15 hours after the capsule had been swallowed.[19] Samples (1 ml) of luminal fluid were collected by siphonage in ice cooled sterile plastic tubes; one was transferred for microbiological analysis and eight were frozen at –70°C for subsequent analysis of microflora associated characteristics. In addition, 0·5 ml of luminal fluid was inoculated in fluid thioglycollate medium for a gas production test.

Microbial cultivation

The homogenised biopsy specimen, the luminal contents, and the saliva were processed for aerobic and anaerobic microbial cultivation within two hours.[20] The samples were suspended in previously reduced peptone yeast extract medium, diluted, and inoculated in various media.[21] The aerobic agar plates were incubated for 24 hours and the anaerobic plates for 48 hours at 37°C in anaerobic jars. Aerobic bacteria were identified biochemically, and anaerobic bacteria were identified by biochemical tests and gas-liquid chromatography.[21]

Micro-organism associated characteristics

The complex interaction between microflora and host was evaluated in samples of luminal fluid principally by the tryptic activity.[22] For the determination of the latter, aliquots of 0·1 ml were added to 2·9 ml TRIS buffer (pH 8·2) containing calcium chloride (4·4 g/l). *N*-Benzoyl-DL-arginine-4-nitroanilide hydrochloride (BAPNA) was added and the reaction (performed at room temperature) was stopped after 10 minutes by adding 0·6 ml 5 M acetic acid. Bovine pancreas trypsin type III was used for the construction of the standard curve. All

samples and standards were analysed spectrophotometrically in parallel with blanks at 405 nm and the tryptic activity was expressed as mg/kg jejunal fluid.

In addition, to detect the presence of microflora with faecal characteristics, the conversion of cholesterol to coprostanol and the presence of urobilinogen and β aspartylglycine was estimated.[22]

Gas production test

Production of gas was measured by placing a Durham tube (inner diameter 5 mm) in a cultivation tube with dehydrated thioglycollate broth (Difco Laboratories, Detroit, USA) containing 0·55% dextrose.[23] Jejunal fluid (0·5 ml) was incubated in the medium and, to create an anaerobic milieu, the pyrogallol method was used.[24] The broth was incubated at 37°C for seven days. Gas production was judged as negative (no gas), trace (1–5 mm gas) or positive (>5 mm gas).

Bile acid deconjugation test

Glycine-1-^{14}C-cholic acid (185 kBq) was administered by mouth, followed immediately by 200 ml orange juice, to fasting sedentary subjects.[25] The subjects delivered breath samples into liquid scintillation vials containing hyamine hydroxide and phenolphthalein at 0, 0·5, 1, 2, 3, and 4 hours after isotope administration. Carbon-14 was measured in a liquid scintillation counter. The output of carbon-14 labelled carbon dioxide was calculated assuming a carbon dioxide production of 9 mmol/kg/hr. Results were expressed as a percentage of administered carbon-14 exhaled over four hours (reference range ≤1·5% of administered dose).

STATISTICAL ANALYSIS

Values are given as medians and first to third quartile (interquartile range). The Kruskal-Wallis test, the Mann-Whitney U test and Fisher's exact probability test were used to test significance. All hypothesis testing was performed at the 0·05 level of significance using two sided tests.

Results

CHARACTERISTICS OF PATIENTS WITH RA AND CONTROLS

Most of the patients with RA had evidence of marked rheumatic disease activity and none of the studied subjects had any gastrointestinal symptoms. All patients with RA were positive for rheumatoid factor (table 2). The median (interquartile range) Riel index was 2·75 (2·50–3·25), the median Ritchie articular index 13 (9–19), and the median HAQ index 1·56 (0·88–2·13). The indices of the patients with RA with hypochlorhydria or achlorhydria were not significantly different from those of the patients with normal acid secretion. Circulating immune complexes were more common in patients with RA with normal acid secretion than in healthy volunteers ($p<0·05$) (table 2). Demographic characteristics and the distribution of the ABO blood group are given in table 1 and biochemical measurements in table 2.

GASTRIC ACID SECRETION

Basal achlorhydria—that is, a basal gastric acid output of 0 mmol/30 minutes, was found in 12/25 (48%) patients with RA and in 3/11 (27%) healthy volunteers (control II). Fifteen of the 25 (60%) patients with RA had a fasting pH>3 compared with 4/11 (36%) healthy volunteers. Stimulated achlorhydria or hypochlorhydria—that is, a peak gastric acid output less than 1·28 mmol/30 minutes, was found in 8/25 (32%) patients with RA, all with fasting pH>6, whereas 17/25 (68%) patients had normal gastric acid secretion with a median peak gastric acid output of 9·1 (5·1–12·7) mmol/30 minutes. Seven of 17 (41%) patients with RA with normal acid secretion had a fasting pH>3. All 11 healthy volunteers had normal gastric acid secretion with a median peak gastric acid output of 14·9 (10·1–20·8) mmol/30 minutes ($p<0·01$ compared with patients with RA with normal gastric acid secretion). All control I subjects had basal and stimulated achlorhydria with a fasting pH>6.

MICROBIOLOGICAL INVESTIGATIONS

A predominance of oral-type microflora was observed in saliva and jejunal contents of patients with RA and controls (fig 1). None of the healthy volunteers had growth of Enterobacteriaceae (*Escherichia coli* or *Klebsiella*) compared with 5/25 patients with RA and 6/12 achlorhydric controls ($p<0·05$ for difference between controls I and II). Similarly, anaerobic oral-type flora was recorded in patients and controls (fig 1).

Table 2 Biochemical and immunological measurements in hypochlorhydric or achlorhydric patients with rheumatoid arthritis (RA), patients with RA and normal gastric acid secretion, achlorhydric controls (control I), and normal healthy volunteers (control II). Values are median (interquartile range) or No (%)

	Reference values	*All patients with RA* (n=25)	*Hypochlorhydric or achlorhydric patients with RA* (n=8)	*Patients with RA with normal gastric acid secretion* (n=17)	*Control I* (n=12)	*Control II* (n=11)
Erythrocyte sedimentation rate (mm/h)	<20	60(33–78)	51(36–61)*	60(29–80)†	6(4–8)	5(4–6)
C reactive protein (mg/l)	<5	35(10–76)	44(5–66)*	35(10–69)†	All <5	All <5
Orosomucoid (mg/l)	0·5–1.0	1·9(1·4–2·3)	2·0(1·4–2·2)*	1·9(1·4–2·2)†	0·8(0·6–0·9)	0·8(0·7–0·9)
Thrombocytes (×10^9/l)	150–400	375(326–486)	450(342–489)*	373(309–406)†	224(204–240)	300(257–317)
Haemoglobin (g/l)	120–170	112(103–125)	114(107–123)*	112(99–124)†	140(134–152)	130(130–142)
Rheumatoid factor (IU)	<20	110(55–415)	100(32–430)*	100(60–198)*	0(0)	0(0)
IgA (g/l)	0·7–3·8	3·0(1·8–3·8)	3·0(2·4–3·2)	3·2(1·8–3·8)	2·8(1·8–3·4)	2·7(1·6–3·7)
Presence of circulating immune complexes	0	13(52)	4(50)	9(53)†	5(42)	1(9)

*p<0·05 compared with achlorhydric patients (control I).
†p<0·05 compared with healthy volunteers with normal gastric acid secretion (control II).

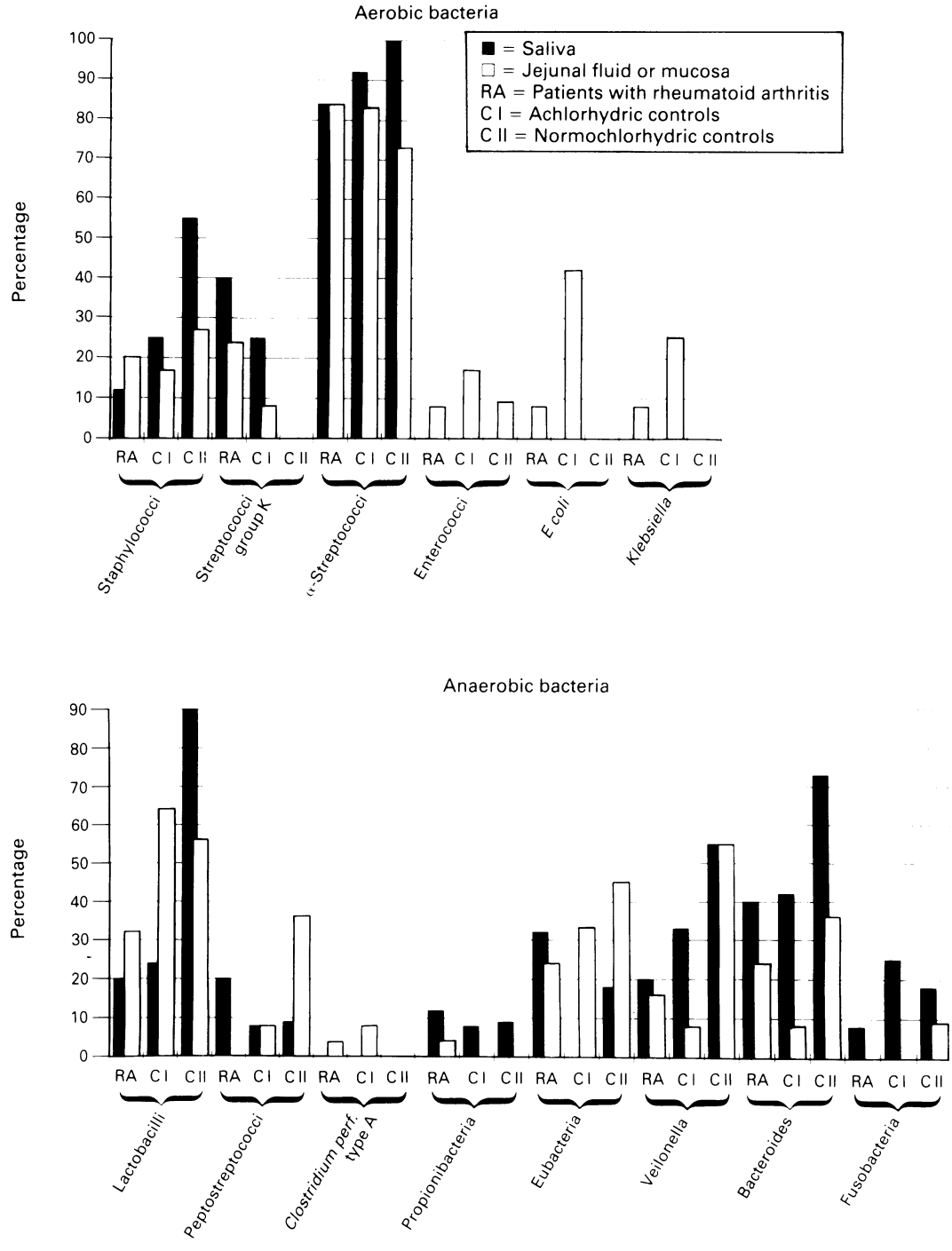

Figure 1 Percentage of patients and controls with positive cultivations for aerobic and anaerobic bacteria in jejunal fluid or mucosa and in saliva. A predominance of oropharyngeal flora is observed in all subjects. Note that Enterobacteriaceae was only found in patients with rheumatoid arthritis and in achlorhydric controls.

MICROFLORA ASSOCIATED CHARACTERISTICS

The median tryptic activity in jejunal contents was 223 (82–282) μg/g in hypochlorhydric or achlorhydric patients with RA and 221 (98–305) μg/g in patients with RA with normal gastric acid secretion. These values were significantly lower than the 298 (283–365) and 729 (335–793) μg/g observed in the achlorhydric controls and the controls with normal gastric acid secretion respectively ($p<0.05$; table 3; fig 2). The achlorhydric controls had significantly lower values than the controls with normal gastric acid secretion. Tryptic activity was not significantly different in patients with RA with normal gastric acid secretion and hypochlorhydric or achlorhydric patients with RA (fig 2).

Coprostanol, urobilinogen, and β aspertylglycine were not detected in the jejunal contents of any of the subjects studied.

GAS PRODUCTION TEST

A gas production test was performed in 20 patients with RA (six hypochlorhydric or achlorhydric and 14 with normal gastric acid secretion), six achlorhydric controls, and eight normal controls. Twenty per cent of the patients with RA (two with normal gastric acid secretion and two hypochlorhydric or achlorhydric patients) and 33% of the achlorhydric controls (2/6) had a positive test result, whereas none of the healthy volunteers (control II) had evidence of pathological gas production (table 3).

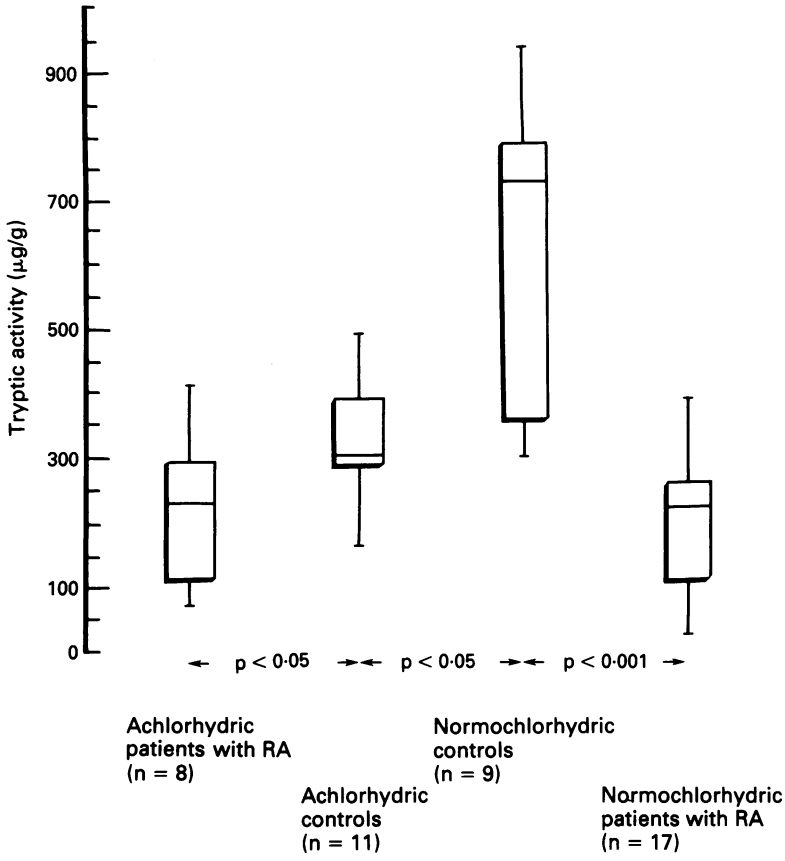

Figure 2 Tryptic activity in jejunal fluid in hypochlorhydric or achlorhydric patients with rheumatoid arthritis (RA), achlorhydric and normochlorhydric controls, and normochlorhydric patients with RA. Values are median, interquartile range (boxes) and 10th and 90th centile (vertical bars). Note that patients with RA had significantly lower tryptic activity than controls and that tryptic activity was significantly lower in achlorhydric controls than in healthy volunteers.

Eighty three per cent of the subjects (5/6) with a positive gas production test result had growth of Enterobacteriaceae. In contrast, enterobacteria were present in only 4% of the subjects with a negative gas production test result.

BILE ACID DECONJUGATION TEST

Twelve per cent (3/25) of the patients with RA and 25% (3/12) of the achlorhydric controls had a pathological finding in the bile acid deconjugation test; none of the healthy volunteers had such a finding (table 3).

BACTERIAL OVERGROWTH, PATIENT CHARACTERISTICS, AND DISEASE ACTIVITY

Patients with positive findings in at least one of the tests described here were considered to have bacterial overgrowth (table 3). There are no reference values for tryptic activity; however, the tryptic activity in patients with RA with evidence of bacterial overgrowth was 94 (64–102) μg/g compared with 259 (184–353) μg/g in patients with normal test values ($p<0·01$). For practical purposes an arbitrary limit of 102 μg/g was therefore used to define patients with a tryptic activity compatible with the presence of abnormal small intestinal microflora.

Forty per cent (10/25) of all the patients with RA had evidence of bacterial overgrowth and in 70% of these two or more tests were positive. Thirty five per cent (6/17) of the patients with RA with normal gastric acid secretion had bacterial overgrowth compared with none of the healthy volunteers ($p<0·05$). The frequency of bacterial overgrowth was similar in hypochlorhydric or achlorhydric patients with RA and in patients with RA with normal gastric acid secretion (table 3). Eight of 15 (53%) patients with RA with fasting pH>3 had bacterial overgrowth compared with 2/10 (20%) patients with fasting pH<3.

There was no significant difference in the duration or dose of NSAID treatment between patients with RA with and without evidence of bacterial overgrowth. In the two groups two patients were not treated with NSAIDs.

The Riel index, the Ritchie articular index, and the HAQ index were significantly higher in patients with RA with bacterial overgrowth than in patients with normal test values (table 4; $p<0·05$). In addition, the rheumatoid factor titres of patients with bacterial overgrowth were 265 (80–570) IU compared with 70 (50–150) IU observed in patients without evidence of abnormal microflora (table 4; $p<0·05$).

The potential influence of genetic factors in the microbiological steady state was simply evaluated by examining the distribution of the blood groups in the studied patients with RA. Patients with RA with blood group O had significantly lower tryptic activity and higher rheumatoid factor titres than patients with blood group A (table 5). Blood group O was more common in patients with RA with bacterial overgrowth (table 5).

Table 3 Bacterial overgrowth in small intestine in hypochlorhydric or achlorhydric patients with rheumatoid arthritis (RA), patients with RA with normal gastric acid secretion, achlorhydric controls (control I), and normal healthy volunteers (control II). Values are median (interquartile range) or No (%)

	Hypochlorhydric or achlorhydric patients with RA (n=6–8)	*Patients with RA with normal gastric acid secretion (n=14–17)*	*Control I (n=6–12)*	*Control II (n=9–11)*
Tryptic activity (μg/g)	223(82–282)*	221(98–305)†	298(283–365)†	729(335–793)
Bacterial overgrowth of small intestine‡	4(50)	6(35)†	6(50)†	0(0)
Growth of Enterobacteriaceae	2	3	6†	0
Tryptic activity ≤102 μg/g	3	5	0	0
Positive gas production test	2	2	2	0
Increased bile acid deconjugation	1	2	3	0

*p<0·05 compared with achlorhydric patients (control I).
†p<0·05 compared with healthy volunteers with normal gastric acid secretion (control II).
‡At least one positive test was required as a criterion of bacterial overgrowth. Patients with a trypic activity ≤102 μg/g were considered to have bacterial overgrowth.

Table 4 Clinical and biochemical disease activity and immunological parameters in patients with rheumatoid arthritis (RA) with or without bacterial overgrowth in the upper small intestine. Values are median (interquartile range) or No (%)

	Patients with RA with bacterial overgrowth (n=10)	*Patients with RA without bacterial overgrowth (n=15)*
Riel index	3·13(2·75–3·25)*	2·75(2·40–3·00)
Ritchie articular index	18(14–26)*	10(7–13)
Health assessment questionnaire	1·81(1·13–2·50)*	1·25(0·81–1·69)
Erythrocyte sedimentation rate (mm/h)	73(40–80)	45(29–63)
C reactive protein (mg/l)	64(26–79)	32(10–63)
Rheumatoid factor (IU)	265(80–570)*	70(50–150)
Presence of circulating immune complexes	6(60)	7(47)

*p<0·05 compared with patients without bacterial overgrowth.

Table 5 Frequency of bacterial overgrowth, tryptic activity, hypochlorhydria or achlorhydria, rheumatoid factor, and circulating immune complexes in 25 patients with rheumatoid arthritis with different ABO blood groups. Values are median (interquartile range) or No (%)

	ABO blood group			
	A (n=8)	*B* (n=5)*	*AB* (n=3)*	*O (n=9)*
Patients with bacterial overgrowth	1(12)	0	3(100)	6(67)†
Patients without bacterial overgrowth	7(88)	5(100)	0	3(33)
Tryptic activity (μg/g)	255(247–282)	221(186–286)	83(74–88)	102(80–133)†
Presence of hypochlorhydria or achlorhydria	3(38)	1(20)	1(33)	3(33)
Rheumatoid factor (IU)	60(45–70)	60(45–180)	430(255–500)	360(110–575)†
Presence of circulating immune complexes	1(13)	3(60)	1(33)	8(89)†

*No comparative statistics were calculated owing to the low number of patients.
†p<0·05 compared with blood group A.

Discussion

This study shows an increased frequency of bacterial overgrowth in the upper small intestine of patients with RA. Although about one third of our patients had hypochlorhydria or achlorhydria, which is associated with small intestinal overgrowth,[14] bacterial overgrowth was also observed in patients with normal acid secretion. This finding indicates that, in addition to hypochlorhydria, other, at present unknown, factors contribute to the development of bacterial overgrowth in patients with RA. Further, it is of great interest that the patients with RA with bacterial overgrowth had a more pronounced disease activity than the patients with normal microflora.

High counts of streptococcal species and Enterobacteriaceae have been recorded in the small intestine of patients with RA,[26] which in the latter case agrees well with our findings. The bacteriological findings in the upper small intestine largely reflected the oropharyngeal microflora; in addition, Enterobacteriaceae were found in the achlorhydric controls and in the patients with RA irrespective of their gastric acid secretion capacity.

The fact that about one third of our patients with RA had hypochlorhydria or achlorhydria reflects the high incidence of atrophic gastritis in those patients.[12 27] The high frequency of bacterial overgrowth recorded in the achlorhydric controls and in hypochlorhydric or achlorhydric patients with RA indicates indirectly that gastric acid secretion is an important defence factor against exogenous pathogenic micro-organisms. The bactericidal activity of gastric juices appears to be pH dependent[28 29] and even a transient reduction of the gastric pH is effective in clearing gastric bacteria, as previously reported in patients with RA in whom cultivations of gastric juices were obtained before and after stimulation with pentagastrin.[13] Consequently, we believe that peak acid output is as reliable as other criteria of hypochlorhydria[30 31] for predicting small intestinal bacterial overgrowth in patients with RA.

Motility disorders, IgA deficiency, clearance of intestinal bacteria by pancreatic and biliary secretions, and the epithelial turnover are known factors that influence the normal steady state of the small intestinal microflora.[32] The influence of these factors on the presence of bacterial overgrowth in patients with normal gastric acid secretion was not investigated, except for the plasma levels of IgA, which were within normal limits. In addition to unknown factors inherent in RA, it can be argued that treatment itself may favour the development of bacterial overgrowth. Studies in humans, however, show that therapeutic doses of NSAIDs accelerate the small intestinal transit time in healthy subjects,[33] which suggests that drug induced small intestinal stasis as a cause of bacterial overgrowth[34] was unlikely in our patients.

Non-steroidal anti-inflammatory drugs increase cell losses from the epithelial surface,[35] and may induce epithelial damage in animals[36] and in humans.[37] In addition, long term treatment with NSAIDs may be associated with inflammatory changes of the distal small intestinal mucosa.[38] It has been suggested that intraluminal bacteria may facilitate the development of some of these changes as they can be reduced by metronidazole[39 40] and as the small intestine of germ free rats is resistant to damage by NSAIDs.[41] These findings do not necessarily imply that NSAIDs give rise to small intestinal bacterial overgrowth, however, and such overgrowth in our patients was not related to the presence, dose, or duration of NSAID treatment.

Gobelet *et al*[42] reported that 35% of a group of patients with RA had abnormal *p*-aminobenzoic acid test results, which may suggest a reduction of exocrine pancreatic function in those patients. The significance of these findings is, however, difficult to evaluate. It is conceivable that such a reduction, if real, may at least partly be related to the high duodenal pH present in patients with RA with hypochlorhydria,[43] which may negatively affect the stimulation of the pancreas. Such factors should, however, not be of importance in our patients with RA and normal acid secretion.

Genetic factors of the host may affect the composition of the indigenous faecal microflora in humans,[44] and also seem to influence the defence mechanisms of the gastroduodenal mucosa in patients with RA.[45 46] In this context, it is of interest that blood group O was over-represented in patients with bacterial overgrowth, which may indicate a genetic predisposition in a subpopulation of patients with RA.

An important observation in this study is that patients with bacterial overgrowth had evidence of severe arthritis and marked disease activity, as evaluated by clinical and biochemical parameters. At present, there is increasing evidence that the intestinal microflora may play a part in triggering or modulating RA,[47–49] as has been shown in adjuvant induced arthritis.[50] In addition, several experimental studies show that bacterial wall products, particularly peptidoglycan-polysaccharide complexes, induce arthritis in animals.[51 52] Thus it has been suggested that changes in the luminal concentration of peptidoglycan or in the permeability of the intestinal mucosa, or both, may be of importance for the development of arthritis. In this context, the fact that our patients with bacterial overgrowth had signs of severe arthritis and pronounced disease activity is of particular interest as patients with RA treated with NSAIDs have increased small intestinal permeability to macromolecules.[53]

A diversity of methods was used to evaluate the presence of bacterial overgrowth, considering that the available techniques disclose different types of bacteria and that some methods are not able to detect metabolic changes in the bacterial population. In addition, great variation in the sensitivity of the tests has been reported in various studies.[54] Thus it seems relevant to define bacterial overgrowth by combining the results obtained by different techniques.[55]

Tryptic activity reflects the net sum of mechanisms involving pancreatic and epithelial cell functions and it can, to some extent, disclose metabolic bacterial changes in the microflora.[56 57] It is of great interest that the tryptic activity in patients with RA with normal gastric acid secretion is compatible with the presence of abnormal bacterial strains, which is further supported by the results obtained when all the techniques were evaluated together.

As discussed elsewhere, gastric acid effectively eliminates luminal bacteria.[13 28 29] Thus the presence of bacterial overgrowth in patients with RA with normal gastric acid secretion and the complete absence of bacteria in the small intestine of the normal controls strongly indicate that possible oropharyngeal contamination due to the prolonged sampling procedure was not of such magnitude as to affect the interpretation of the data of this mainly qualitative microbiological study.

In conclusion, the high frequency of small intestinal bacterial overgrowth found in patients with RA was associated with a high disease activity and it was observed in patients with hypochlorhydria or achlorhydria and those with normal gastric acid secretion.

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